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Synthesis and Characterization of New Diketone Analogues of Podophyllotoxin

K. H. HEMAKUMAR*, A. D. SATHISHA and Y. B. BASAVARAJU

Department of Studies in Chemistry,
University of Mysore, Manasagangotri, Mysore-570 006, Karnataka, India.

basavaraju_yb@yahoo.co.in

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Abstract: The new compounds 6,6a-dihydro-2,3-dimethoxy-9-nitro-11bH benzo [C]-fluoren 5,7-dione, 6,6a-dihydro-2,3-dimethoxy-9-chloro-11bH benzo[C]-fluoren-5,7-dione and 6,6a-dihydro-2,3-dimethoxy-9-fluoro-11bH benzo[C]-fluoren-5,7- dione were synthesized in high yields. They are analogues of naturally occurring lignan podophyllotoxin which exhibits anticancer activity. They are very essential to study anticancer activity.

Keywords: Benzophenones, Stobbe condensation, Itaconic acids, Sodium-amalgam, Benzhydryl succinic acids, Diketones.

Introduction

Podophyllotoxin **1** is a naturally occurring lignan compound, which has been isolated from the plants of genus *Podophyllum*¹⁻⁴ belongs to the family of *Berberidaceae*. As the compound **1** was found to be highly cytotoxic for its clinical use against human cancers⁵, extensive structural modifications of **1** have been undertaken which culminated in to two semi-synthetic analogues of podophyllotoxin, namely etoposide (VP-16) and tenoposide (VM-26) are now in clinical use. Several analogues of podophyllotoxin have been synthesized with a view to study their structure activity relationship⁶. Hence, it was decided to synthesize analogues **2**, **3** & **4** by modifying the structure of podophyllotoxin **1**. Several synthetic routes⁷ have been reported for the synthesis of analogues of podophyllotoxin **1**. In this context, we have chosen Gensler's^{8,9} method with some changes in the experimental procedure and reagents to synthesize diketone analogues.

Experimental

Melting points of the products were determined by the open capillary method and are uncorrected. The IR spectra were recorded on a FT-IR in KBr disc or in nujol mull. The ¹H NMR spectra were recorded on Jeol-60MHz and Jeol GSX 400MHz spectrophotometer

using CDCl_3 as a solvent and TMS as an internal reference. The chemical shifts are expressed in δ (ppm) values. The Mass spectra were recorded on Hitachi RMU-61 spectrophotometer and important fragments are given with percentage of abundance in the bracket. The purity of the compounds were checked by thin layer chromatography on silica gel glass plates in benzene and ethyl acetate solvent mixture (7:0.5 v/v). The compounds were purified by column chromatography using silica gel (60-120 mesh) as adsorbent and benzene as eluent.

General procedure for the preparation of 3,4-dimethoxy-4'-nitro-benzophenone (7a)

Veratrole (**5**) (10g, 0.0724mole) and anhydrous aluminum chloride (9.650g, 0.0724mole) were taken in dichloromethane (75mL). The reaction mixture was cooled to 0°C and protected from atmospheric moisture. It was stirred continuously for 30min. A solution of *p*-nitro benzoyl chloride (13.43g, 0.0724mole) in dichloromethane (75mL) was added drop wise over a period of 1 h to the above reaction mixture. After 12 h, the temperature of the reaction mixture had been allowed to come to 25°C , conc. HCl (54mL) was added drop wise over a period of 30min. The reaction mixture was further stirred for 10h. During the addition of HCl and for some time thereafter, large amount of HCl gas is evolved. The product was extracted into chloroform, washed with 10% aqueous NaOH solution (2x100mL) and then with 2% aqueous NaCl solution (2x75mL). The solvent was removed by distillation. The product was recrystallized from methanol to give brown crystalline compound in 72.96% yield (15.3g). M.p. $143\text{--}145^\circ\text{C}$. IR (KBr): 1680 (C=O), 1596 (aromatic C=C) cm^{-1} ; ^1H NMR (CDCl_3): δ 3.9 (s, 6H, OCH_3), 6.8 (m, 3H, C_5 , C_3 & $\text{C}_5\text{-H}$), 7.3 (m, 4H, C_2 , C_6 , C_2' & $\text{C}_6\text{-H}$); Anal. Calcd. for $\text{C}_{15}\text{H}_{13}\text{O}_5\text{N}$: C, 62.72; H, 4.56; N, 5.22 %; Found: C, 62.70; H, 4.50; N, 5.19 %.

3,4-Dimethoxy-4'-chloro-benzophenone (7b)

Prepared from veratrole (**5**) (10g, 0.0724mole) and *p*-chloro benzoyl chloride (10.10g, 0.0724mole) as yellow semisolid compound in 74% yield (14.93g). IR (Nujol): 1684 (C=O), 1594 (aromatic C=C) cm^{-1} ; ^1H NMR (CDCl_3): δ 3.9 (s, 6H, OCH_3), 6.7 (m, 3H, C_5 , C_3 & $\text{C}_5\text{-H}$), 7.2 (m, 4H, C_2 , C_6 , C_2' & $\text{C}_6\text{-H}$); Anal. Calcd. for $\text{C}_{15}\text{H}_{13}\text{O}_3\text{Cl}$: C, 65.10; H, 4.73%; Found: C, 65.05; H, 4.70 %.

3,4-Dimethoxy-4'-fluoro-benzophenone (7c)

Prepared from veratrole (**5**) (10g, 0.0724mole) and *p*-fluoro benzoyl chloride (8.91g, 0.0724mole) as buff coloured solid in 76% yield (14.31g). M.p. $102\text{--}104^\circ\text{C}$. IR (KBr): 1678 (C=O), 1599 (aromatic C=C) cm^{-1} ; ^1H NMR (CDCl_3): δ 3.8 (s, 6H, OCH_3), 6.9 (m, 3H, C_5 , C_3 & $\text{C}_5\text{-H}$), 7.3 (m, 4H, C_2 , C_6 , C_2' & $\text{C}_6\text{-H}$); Anal. Calcd. for $\text{C}_{15}\text{H}_{13}\text{O}_3\text{F}$: C, 69.22; H, 5.03%; Found: C, 69.20; H, 5.00%.

General procedure for the preparation of 3,4-dimethoxy-4'-nitro-diphenyl itaconic acid (9a)

It is prepared by the Stobbe condensation of 3,4-dimethoxy-4'-nitro benzophenone (**7a**) (15.0g, 0.0522mole) with diethyl succinate (9.09g, 0.0522mole) in presence of potassium *t*-butoxide (obtained from potassium 2.04g, 0.0522mole and *t*-butyl alcohol) in *t*-butyl alcohol (100mL) at reflux temperature for 10h. The cooled reaction mixture was treated with 5N conc. HCl (50mL) was concentrated to 60mL and diluted with water (75mL). The itaconic acid half esters were extracted into ether (3x50mL) and then into saturated sodium bicarbonate solution (3x50mL). The bicarbonate solution was acidified with conc. HCl to give a brown crystalline solid in 83.9% yield (18.2g). It was recrystallised from ethanol to give a pale brown crystalline solid. M.p. $97\text{--}98^\circ\text{C}$. The itaconic acid half esters were saponified by refluxing in methanol (50mL) and water (50mL) mixture containing NaOH (6g). The reaction

mixture was acidified with conc. HCl to give a grayish white solid. It was recrystallized from methanol to give white solid in 86% yield (14.43g). M.p. 91-93 °C. IR (KBr) : 3600-3300 (Carboxylic OH), 1700 (-CH₂-C=O), 1680 (α , β - unsaturated C=O), 1590 (aromatic C=C), 1610-1605 (conjugated C=C) cm⁻¹; ¹H NMR (CDCl₃): δ 3.9 (s, 6H, OCH₃) , 3.6 (s, 2H, CH₂), 6.7 (m, 3H, C₃, C₅' & C₅-H), 7.1 (m, 4H, C₂, C₆, C₂' & C₆-H); Anal. calcd. for C₁₉H₁₇O₈N : C, 58.91; H, 4.42; N, 3.87 %; Found: C, 58.89; H, 4.40; N, 3.83%.

3,4-Dimethoxy-4'-chloro-diphenyl itaconic acid (9b)

Prepared as white crystalline solid in 82% yield (12.97g). M.p. 158 °C, by the Stobbe condensation of 3,4-methoxy-4'-chloro benzophenone (**7b**) (14g, 0.0506mole) with diethyl succinate (8.81g, 0.0505mole) followed by saponification. IR (KBr): 3560-3330 (carboxylic OH), 1698 (-CH₂-C=O), 1672 (α , β -unsaturated C=O), 1598 (conjugated C=C), 1593 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃): δ 3.8 (s, 6H, OCH₃), 3.7 (s, 2H, CH₂), 6.8 (m, 3H, C₃, C₅, & C₅-H), 7.2 (m, 4H, C₂, C₆, C₂' & C₆-H); Anal. calcd. for C₁₉H₁₇O₆Cl: C, 60.56; H, 4.54%; Found: C, 60.53; H, 4.50%.

3,4-Dimethoxy-4'-fluoro-diphenyl itaconic acid (9c)

Prepared as white solid in 81.8% yield (12.9g). M.p. 136 °C by the Stobbe condensation of 3,4-methoxy-4'-fluoro benzophenone (**7c**) (14g, 0.0538mole) with diethyl succinate (9.37g, 0.0538mole) followed by saponification. IR (KBr): 3500-3260 (carboxylic OH), 1592 (conjugated C=C), 1603 (aromatic C=C), 1693 (-CH₂-C=O), 1680 (α , β -unsaturated C=O) cm⁻¹; ¹H NMR (CDCl₃): δ 3.9(s, 6H, OCH₃), 3.6 (s, 2H, CH₂-), 6.7 (m, 3H, C₃, C₅' & C₅-H), 7.3 (m, 4H, C₂, C₆, C₂' & C₆-H); Anal. calcd. for C₁₉H₁₇O₅F: C, 63.32 ; H, 4.76%; Found: C, 63.29; H, 4.74%.

General procedure for the preparation of 3,4-dimethoxy-4'-nitro benzhydryl succinic acid (10a)

Powdered 5% sodium-amalgam (200g) was added to a solution of **9a** (14.07g, 0.0361 mole) in 5% aq. NaOH (200mL) solution around 5 °C. The reaction mixture was kept overnight at room temperature and filtered. The filtrate was acidified with 5N HCl gave gray solid. It was recrystallized from ethanol gave white solid in 81% yield (11.39g). M.p. 123-125 °C. IR (KBr) :3500-3200 (carboxylic OH), 1710 (carboxyl C=O), 1590 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃): δ 3.9(s, 6H, OCH₃), 3.0 (d, J= 6Hz, 2H, C_c-H), 3.5 (q, J=6Hz, 1H, C_b-H), 4.1 (d, J=6Hz, 1H, C_a-H), 6.7 (m, 3H, C₃, C₅' & C₅-H), 7.3 (m, 4H, C₂, C₆, C₂' & C₆-H); Anal. calcd. for C₁₉H₁₉O₈N: C, 58.61; H, 4.92; N, 3.85%; Found: C, 58.59; H, 4.90; N, 3.80%.

3,4-Dimethoxy-4'-chloro benzhydryl succinic acid (10b)

Obtained from the reduction of 3,4-dimethoxy-4'-chloro diphenyl itaconic acid **9b** (12g, 0.0318) and 5% sodium-amalgam (200g) as white solid in 79% yield (9.53g). M.p. 117-119 °C. IR (KBr) :3500-3250 (carboxylic OH), 1705 (carboxyl C=O), 1598 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃): δ 3.9 (s, 6H, OCH₃), 2.9 (d, J= 5Hz, 2H, C_c-H), 3.3 (q, J=5Hz, 1H, C_b-H), 4.0 (d, J=5Hz, 1H, C_a-H), 7.2 (m, 4H, C₂, C₆, C₂' & C₆-H), 6.6 (m, 3H, C₃, C₅' & C₅-H); Anal. calcd. for C₁₉H₁₉O₆Cl: C, 60.24; H, 5.05%; Found: C, 60.19; H, 5.02%.

3,4-Dimethoxy-4'-fluoro benzhydryl succinic acid (10c)

Obtained from the reduction of 3,4-dimethoxy-4'-fluoro diphenyl itaconic acid **9c** (12g, 0.0333mole) and 5% sodium-amalgam (200g) as white amorphous solid in 82.4% yield (9.94g). M.p. 110-112 °C. IR (KBr) :3600-3200 (carboxylic OH), 1700 (carboxyl C=O), 1610 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃): δ 3.9 (s, 6H, OCH₃), 2.8 (d, J= 6Hz, 2H, C_c-H), 3.4 (q, J= 6Hz,

1H, C₆-H), 4.1 (d, J= 6Hz, 1H, C_a-H), 7.3 (m, 4H, C₂,C₆,C_{2'} & C₆-H), 6.7 (m, 3H, C₃,C₅ & C₅-H); Anal.calcd. for C₁₉H₁₉O₆F: C, 62.97; H, 4.73%; Found: C, 62.94; H, 4.69%

General procedure for the preparation of 6,6a-dihydro-2,3-dimethoxy-9-nitro-11bH benzo [C]-fluoren-5,7-dione (2)

A mixture of 3,4-dimethoxy-4'-nitro benzhydryl succinic acid **10a** (2.0 g, 0.0051mole) and thionyl chloride (40mL) was refluxed for 5h. The excess thionyl chloride was distilled off. A pale yellow solid was obtained as gummy product **11a** in 80% yield (1.74g). A solution of 3,4-dimethoxy-4'-nitro benzhydryl succinyl chloride **11a** (1.6g, 0.0038mole) in dry dichloromethane (50mL) was added over a period of 20min to a stirred solution of anhydrous aluminium chloride (0.50g, 0.0037mole) in dry dichloromethane at 0 °C. The reaction mixture was further stirred at 0 °C for 6h. After the reaction, the reaction mixture was treated with cold 5N HCl (50mL). The organic layer was washed with 10% NaOH solution (2x50mL) and finally with water. The solvent was removed by distillation to get a brown solid. The crude product was column chromatographed over silica gel (1cmx30cm) using chloroform as the eluent. The solvent was removed at 50 °C on a rotary evaporator to get a pale brown solid. It was recrystallized from ethanol gave 72.3% yield (0.96g). M.p. 163-164 °C. IR (KBr): 1743 (Indonone carbonyl), 1704 (tetralone carbonyl), 1594 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃): δ 2.5 (dd, J=4Hz, 2H, C₆-H), 3.4 (q, J=4Hz, 1H, C_{6a}-H), 3.8 (s, 6H, OCH₃), 4.2 (d, J=6Hz, 1H, C_{11b}-H), 7.5 (s, 1H, C₈-H), 7.3 (s, 1H, C₄-H), 6.7 (m, 3H, C₁-H, C₁₀-H & C₁₁-H); Mass (m/z % abundance): 353 (M⁺, 24), 325 (46), 298 (27), 270 (33), 135 (69), 89 (93). Anal. Calcd. for C₁₉H₁₄O₆N: C, 63.74; H, 3.99; N, 4.24%; Found: C, 63.69; H, 3.97; N, 4.20%.

6,6a Dihydro-2,3-dimethoxy-9-chloro 11bH benzo [C]-fluoren-5,7-dione (3)

Prepared from 3,4-dimethoxy-4'-fluoro benzhydryl succinic acid **10b** (2.0g, 0.0038mole) thionyl chloride (40mL) as brown semisolid **11b** in 78.2% yield (1.70g). The **11b** (1.60g 0.0038mole) was cyclised to **3** using anhyd. aluminium chloride (0.52g, 0.0038mole) in dry dichloromethane (50mL) to get pale yellow semi solid compound in 67% yield (0.87g). IR (KBr): 1740(Indanone C=O), 1696 (tetralone C=O), 1598 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃): δ 2.7 (dd, J= 4Hz, 2H, C₆-H), 3.4 (q, J= 4Hz, 1H, C_{6a}-H), 3.8 (s, 6H, OCH₃), 4.2 (d, J= 4Hz, 1H, C_{11b}-H), 7.4 (s, 1H, C₈-H), 7.2 (s, 1H, C₄-H), 6.7-7.1 (m, 3H, C₁-H, C₁₀-H & C₁₁-H); Mass (m/z % of abundance): 342.5 (M⁺, 19), 307 (26), 279 (44), 252 (37), 224 (32), 89 (76); Anal. Calcd. for C₁₉H₁₄O₄Cl: C, 66.48; H, 4.16%; Found: C, 66.44; H, 4.13%.

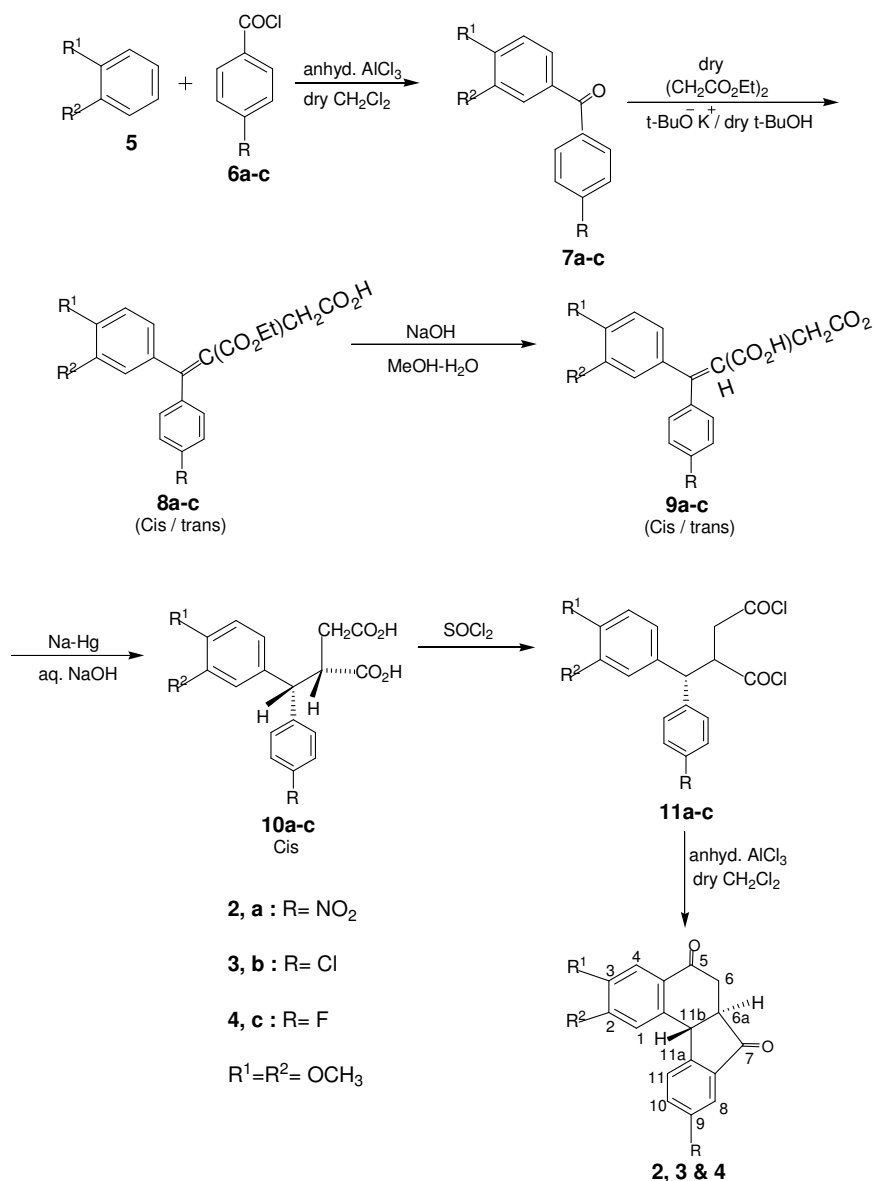
6,6a, Dihydro-2,3-dimethoxy-9-fluoro-11bH benzo [C]-fluoren-5,7-dione 4

Prepared from 3,4-dimethoxy-4'-fluoro benzhydryl succinic acid **10c** (2.0g, 0.0055mole) and thionyl chloride (40mL) as brown semi solid **11c** in 74.1% yield (1.62g). The **11c** (1.6g, 0.0040mole) was cyclised to **4** using anhyd. aluminium chloride (0.54g, 0.0040mole) as catalyst in dry dichloromethane (50mL) gave pale brown semi solid compound in 59.7% yield (0.78 g). IR (KBr):1743 (Indanone C=O), 1693 (tetralone C=O), 1606 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃): δ 2.6 (dd, J= 4Hz, 2H, C₆-H), 3.5 (q, J= 4Hz, 1H, C_{6a}-H), 3.9 (s, 6H, OCH₃), 4.0 (d, J= 4Hz, 1H, C_{11b}-H), 7.7 (s, 1H, C₈-H), 7.3 (s, 1H, C₄-H), 6.8 (m, 3H, C₁-H, C₁₀-H & C₁₁-H); Mass (m/z % of abundance): 326 (M⁺, 23), 298 (37), 271 (43), 243 (17), 108 (54), 89 (83); Anal. Calcd. for C₁₉H₁₄O₄F: C, 69.22; H, 4.33%; Found: C, 69.19; H, 4.29 %.

Results and Discussion

The starting materials benzophenones **7a-c** Were prepared in high yields by Friedel-Crafts acylation of veratrole with acyl chlorides **6a-c** in the presence of anhydrous aluminum

chloride in dry dichloromethane at 0 °C. Itaconic acid half esters **8a-c** were prepared as a mixture of cis and trans isomers in good yields by Stobbe condensation of benzophenones **7a-c** with diethyl succinate using potassium t-butoxide as a base in *t*-butanol at 90 °C (Scheme-1).



Scheme 1

Itaconic acids **9a-c** were prepared in excellent yield by the saponification of itaconic acid half esters **8a-c**. The separation of isomeric mixture by fractional crystallization from ethyl acetate-petroleum ether (60-80) mixture was unsuccessful. Hence, benzhydryl succinic acids

10a-c were prepared by the reduction¹⁰ of isomeric mixture of itaconic acids **9a-c** with 5% sodium-amalgam in 5% aq. sodium hydroxide solution. After the usual workup, only one required benzhydryl succinic acid isomer¹⁰ is formed in good yield. The compounds **11a-c** were prepared by refluxing benzhydryl succinic acids **10a-c** with thionyl chloride. Diketones **2**, **3** & **4** analogues of podophyllotoxin were prepared in good yields by the intramolecular cyclisation of benzhydryl succinyl chlorides **11a-c** in the presence of anhydrous aluminium chloride in dichloromethane. The products were characterized by IR, ¹H NMR and mass spectral and elemental analysis data. The proton signals of carboxylic acids are not observed due to out of scale absorption (δ 10.5-12.0). However, the acid functional groups were characterized by simple chemical tests. The NMR spectra of diketone **2** showed a doublet at δ 4.1 ppm ($J = 6\text{Hz}$) for the benzylic proton C₄-H. The large coupling constant (J) value indicated that C_{6a}-H and C_{11b}-H were diaxial. Hence, the two ketone rings are trans fused and configuration being thermodynamically stable.

Conclusion

In the above synthetic scheme, the products are formed in good yields using less expensive and readily available chemicals. The double bond of the α , β -unsaturated acids can be easily reduced to benzhydryl succinic acids. Benzhydryl succinyl chlorides undergo readily Friedel-Crafts intramolecular cyclisation in the presence of anhyd. aluminium chloride in dry dichloromethane gave high yields of diketone analogues of podophyllotoxin. They are required for studying anticancer activity.

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